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<http://dx.doi.org/10.1289/ehp.1307861>

Received: 7 November 2013

Accepted: 1 August 2014

Advance Publication: 5 August 2014

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Running title: Metal exposure and vitamin D concentrations

Acknowledgments: The investigators thank the Coahuila State Department of Health for providing facilities for the conduct of the study. The study was funded by NIEHS R01ES015597.

Competing financial interests: None.

Abstract

Background: Limited data suggest that lead (Pb), cadmium (Cd) and uranium (U) may disrupt vitamin D metabolism and inhibit production of 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$), the active vitamin D metabolite, from 25-hydroxyvitamin D ($25(\text{OH})\text{D}$) in the kidney.

Objectives: To evaluate the association between blood lead (BPb) and urine arsenic (As), Cd, molybdenum (Mo), thallium (Tl) and U with markers of vitamin D metabolism ($25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$).

Methods: We conducted a cross-sectional study of 512 adolescents in Torreon, a town in Mexico with a Pb smelter near residential areas. BPb was measured using atomic absorption spectrometry. Urine As, Cd, Mo, Tl, and U were measured using inductively coupled plasma mass spectrometry. Serum $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$ were measured using a chemiluminescent immunoassay and a radioimmunoassay, respectively. Multivariable linear models with vitamin D markers as the outcome were used to estimate associations of blood lead and creatinine-corrected urine As and metal concentrations with serum vitamin D concentrations, controlling for age, sex, adiposity, smoking, SES, and time outdoors.

Results: Serum $25(\text{OH})\text{D}$ was positively associated with urine Mo and Tl [1.5 (95% CI: $0.4, 2.6$) and 1.2 (95% CI: $0.3, 2.1$) ng/ml higher with a doubling of exposure, respectively]. Serum $1,25(\text{OH})_2\text{D}$ was positively associated with urine As and U [3.4 (95% CI: $0.9, 5.9$) and 2.2 (95% CI: $0.7, 3.7$) pg/mL higher, respectively], with little change in associations after additional adjustment for serum $25(\text{OH})\text{D}$. Pb and Cd were not associated with $25(\text{OH})\text{D}$ or $1,25(\text{OH})_2\text{D}$ concentrations.

Conclusions: Overall, our findings did not support a negative effect of As or metal exposures on serum $1,25(\text{OH})_2\text{D}$ concentrations. Additional research is needed to confirm positive associations

between serum $1,25(\text{OH})_2\text{D}$ and urine U and As concentrations, and clarify potential underlying mechanisms.

Introduction

Vitamin D is a steroid hormone produced in the skin upon exposure to ultraviolet B (UVB) radiation. UVB stimulates the conversion of 7-dehydrocholesterol into vitamin D, which is then hydroxylated in the liver to 25(OH)D and further hydroxylated in the kidney to 1,25(OH)₂D, the active form of vitamin D (Holick 2007, DeLuca 2004). Vitamin D deficiency may play a role in susceptibility to several chronic diseases, including atherosclerosis, hypertension, asthma, certain cancers, and certain autoimmune diseases (Brondum-Jacobsen et al 2012, Giovannucci et al 2006, Holick 2004, Holick 2007, IOM 2011, Ponsonby et al 2002). Vitamin D status is influenced by many factors, including sun exposure, adiposity, genetics, skin complexion, geographic latitude, time of year, and age (Holick 2004, Holick 2007). There is also interest in identifying environmental factors that may influence vitamin D status.

A limited number of studies suggest that exposure to toxic metals may influence vitamin D status. Higher blood lead concentrations in children were associated with higher 25(OH)D in one study (Kemp et al 2006) and with lower 1,25(OH)₂D in a different study, leading to the hypothesis that Pb could inhibit the production of 1,25(OH)₂D in the kidney (Rosen et al 1980). Cadmium (Cd) exposure has also been associated with lower 1,25(OH)₂D concentrations (Nogawa et al 1987, Nogawa et al 1990) but similar 25(OH)D concentrations compared to unexposed individuals (Nogawa et al 1990). In addition, experimental studies in rats reported that uranium (U) exposure decreased 1,25(OH)₂D concentrations (Tissandie et al 2006, Tissandie et al 2007) with no change in 25(OH)D concentrations (Tissandie et al 2007).

In the present study, we evaluated the association of biomarkers of exposure to arsenic (As) and metals with 25(OH)D and 1,25(OH)₂D concentrations in a population of adolescent boys and

girls from a community in Northern Mexico exposed to lead and cadmium emitted by a lead/zinc smelter complex (Albalak et al, 2003) as well as to arsenic and other metals *via* drinking water (Del Razo et al, 1993). We hypothesized that higher concentrations of BPb and urine Cd, and potentially other trace elements, would be associated with lower concentrations of 1,25(OH)₂D.

Methods

Participants were recruited in the city of Torreon in northern Mexico. Torreon is the home of the Met-Mex Penoles smelter complex, located in the southern area of the city and surrounded by residential areas (Garcia-Vargas et al 2014, Albalak et al 2003, Benin et al 1999). In addition, the groundwater used for drinking in much of the area contains elevated concentrations of As, up to 50 µg/L or higher (Camacho et al 2011). Study participants were sampled among participants in an earlier census for biomonitoring BPb concentrations conducted by the State of Coahuila Department of Health since 1999. The study population consisted of boys and girls 12-15 years of age at the time of recruitment (October 2009 to June 2010) who had participated in this earlier census and for whom there was at least one BPb measurement prior to 2004. Census participants were divided into five strata according to prior BPb concentrations, and participants were randomly chosen from each stratum until the pre-specified sample size of 512 participants was reached, designed to have 90% power to detect effect sizes ≥ 0.15 associated with a doubling of the concentration of the heavy metal biomarkers.

The study was approved by the Institutional Review Boards of Juarez University of Durango State, the Johns Hopkins Bloomberg School of Public Health, and the New York State Department of Health. All participants, as well as their parents or legal guardians, gave signed, informed consent.

Study data were collected by trained personnel in two home visits and a visit to the study clinic. Questionnaire data included information on family structure and income, diet, physical activity, academic history, exposure history of family members to Pb, and household smoking history. As a marker of adiposity, we measured percent fat mass using impedance as measured on a Tanita scale (Model BC-418, Tokyo, Japan). We also obtained a fasting blood specimen and a spot urine specimen from each participant. Blood was collected in EDTA purple top tubes (Becton Dickinson, Franklin Lakes, NJ). Urine was collected in plastic containers that were washed with 10% HNO₃ overnight and rinsed with deionized water. Blood and urine specimens were refrigerated immediately and taken on the same day to the Laboratory of Toxicology at the Juarez University of Durango State where they were aliquoted and frozen at -80°C. More details of these methods have been published elsewhere (Garcia-Vargas et al, 2014).

Pb was measured in whole blood using a graphite furnace atomic absorption spectrometer equipped with Zeeman background correction at the Environmental Toxicology Laboratory at Juarez University of Durango State. All samples with a coefficient of variation greater than 5% were re-analyzed. The limit of detection was 0.7 µg/dL and the average CV was 3.9%. Bovine blood was used as a reference standard.

Urine Cd, As, U, molybdenum (Mo), and thallium (Tl) were measured at the Trace Elements Section at the Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health in Albany, New York. The analyses were carried out using a Perkin Elmer ELAN DRC II inductively coupled plasma-mass spectrometer (ICP-MS) equipped with Dynamic Reaction Cell (DRC-) technology (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, USA). The limits of detection for As, Cd, Mo, Tl, and U were 1.1, 0.02, 1.0, 0.02, and 0.001 µg/L, respectively. Urine Cd values were corrected for the polyatomic interference

from Mo using a procedure previously validated and based on each individuals's Mo value. The details of these laboratory methods have been described elsewhere (Pollack et al 2013). Arsenic was not speciated and is reported as total arsenic. More details have been published elsewhere (Garcia-Vargas et al, 2014).

Urine arsenic and metal concentrations were corrected for urine creatinine concentrations to account for variability in dilution in spot urine specimens. Urine creatinine concentrations were measured using a Dimension clinical chemistry system using a Flex reagent cartridge in an enzymatic assay (Siemens Dimension Vista 1500; Siemens Medical Solutions USA, Inc., Malvern, PA, United States).

There were several reasons for choosing the exposures to measure in this study. We wanted to expand upon prior research on vitamin D and Pb, Cd, and U. Although we did not identify prior research on vitamin D and As, we chose to include As in this study because of the high exposure to As through drinking water. Finally, the method of analysis generates the data for all of these metals at the same time, so we included them as an exploratory analysis since they were available.

Vitamin D metabolites were measured in serum at Heartland Assays, a commercial vitamin D laboratory located in Ames, Iowa. Total 25(OH)D was measured using a chemiluminescent immunoassay on a Diasorin Liaison analyzer. 1,25(OH)₂D was measured using radioimmunoassay. The details of these assays have been published elsewhere (Hollis et al 2007). The coefficient of variation for a random sample of aliquots used as blind duplicates (n=20) was 8.0% for 25(OH)D and 16.3% for 1,25(OH)₂D.

Statistical analyses were conducted using Stata 12 (Stata Corp, College Station, TX). Statistical significance was defined as $p \leq 0.05$. As and metal concentrations were log base 2-transformed to correct for observed skewed distributions. Multiple linear regression models were performed to assess the relationship of each vitamin D metabolite (outcome) with As and metals modeled either as log₂-transformed continuous variables or as quartiles (predictors). We ran three models with progressive adjustment factors. First we ran a model adjusted for age (continuous) and sex. Then, we ran a second model that added season (January-March, April-May, and October-December), household income (≥ 3000 pesos/month, < 3000 pesos/month, and unknown), smoking (never smokers, former smokers, and current smokers), percent fat mass (continuous), and time spent walking outside (none, < 30 minutes/week, 30 minutes – 2 hours/week, 2-4 hours/week, 4-6 hours/week, and > 6 hours/week). Time spent walking was included as a measure of time spent outside, and therefore, sun exposure, which is a potential confounder. While we did not ask participants to report their sun exposure, we collected information on outdoor activities including soccer, bicycling, and skateboarding, however time spent walking was the only variable that predicted 25(OH)D concentrations. Therefore, we used time spent walking as a measure of sun exposure. An additional model used 1,25(OH)₂D as the outcome variable with 25(OH)D as a predictor, since 25(OH)D is a precursor to 1,25(OH)₂D. For exploratory purposes we also conducted stratified analyses by sex, and we included models analyzing U and As in the same model. In the models using quartiles, p-trend was calculated by modeling the quartiles, coded as 1, 2, 3, and 4, as a continuous variable.

As a sensitivity analysis, we reanalyzed the data after natural log-transforming 25(OH)D and 1,25(OH)₂D since the concentrations of vitamin D biomarkers were slightly right skewed. In addition, we repeated the analyses for As after excluding participants who reported eating fish in

the previous week to eliminate the influence of arsenobetaine and other organic arsenic compounds present in fish (Navas-Acien et al 2011). We also adjusted for milk consumption by including a variable where participants reported how many glasses of milk they consume (never, less than one per month, 1-3 per month, one per week, 2-4 per week, 5-6 per week, one per day, 2-3 per day, 4-5 per day, or 6 or more per day), since milk is fortified with vitamin D. Food intake was assessed using the validated Mexican version of Willett's food frequency questionnaire (Hernandez-Avila et al, 1998). We also repeated the analyses using urine measurements corrected for osmolality instead of creatinine. Urine osmolality concentrations were measured with an osmometer utilizing the freezing point depression method (Model 3250; Advanced Instruments, Inc., Norwood, MA). Finally, to test for influential outliers, we used added-variable plots, conducted using the `avplot` command in Stata, to identify outliers and then reanalyzed the data excluding these outliers.

Results

The mean (\pm standard deviation) age of study participants was 14.0 ± 1.2 years. 262 participants (51.2%) were male (Table 1). The mean serum 25(OH)D concentration was 24.8 ± 8.2 ng/mL and mean serum 1,25(OH)₂D concentration was 58.6 ± 18.3 pg/mL. The Spearman correlation coefficient between both vitamin D metabolites was 0.21 ($p < 0.001$). Mean 25(OH)D and 1,25(OH)₂D were higher in males than females (data not shown). Spearman correlations showed higher 25(OH)D concentrations were significantly associated with lower adiposity and more time spent outside, while higher 1,25(OH)₂D concentrations were significantly associated with lower adiposity, but not with time spent outside (data not shown). Of all the trace elements measured, As and U were the most highly correlated ($r = 0.56$, $p < 0.01$) (see Supplemental Material, Table S1).

There was no evidence for an association between BPb or urine concentrations of Cd with 25(OH)D or 1,25(OH)₂D, either using log₂-transformed creatinine-corrected urine metal concentrations as continuous variables or categorized in quartiles (Tables 2-3). Urine Tl and Mo concentrations were positively and significantly associated with 25(OH)D. Urine Mo was positively associated with 1,25(OH)₂D and urine Tl was negatively associated with 1,25(OH)₂D, however these associations were not statistically significant. Urine As was positively and significantly associated with 1,25(OH)₂D but not with 25(OH)D.

Urine U was the only element with a statistically significant association with both vitamin D metabolites, although the association between U and 25(OH)D was not significant after adjusting for season. In multivariable-adjusted models, mean serum 1,25(OH)₂D concentrations were 2.2 pg/mL higher (95% CI: 0.7, 3.7; $p = 0.01$) in association with a doubling of the creatinine-corrected urine U concentration. After additional adjustment for 25(OH)D, mean serum 1,25(OH)₂D concentrations were 2.1 pg/mL higher (95% CI: 0.6, 3.6; $p = 0.01$) with each doubling of urine U concentration.

After excluding the 63 participants who reported eating fish in the past week, the models were repeated for arsenic, with no change in the associations seen (see Supplemental Material, Table S2). There was no significant association with 25(OH)D, while mean 1,25(OH)₂D concentrations were 4.0 pg/mL higher (95% CI: 1.2, 6.7; $p = 0.01$) with a doubling of As. There was no change in the associations after excluding 3 outliers, indicating that these outliers were not influential. There was also no change in any of the associations after adjusting for milk consumption or when adjusting for urine dilution with osmolality instead of urine creatinine, or after natural log-transforming both 25(OH)D and 1,25(OH)₂D.

Creatinine-corrected urine U and As concentrations were highly correlated (Spearman correlation coefficient 0.56, $p < 0.001$) (Supplemental Material, Table S1), consistent with evidence that groundwater contamination is a common source of both exposures (Li, 2005). When U and As were included simultaneously as predictors of vitamin D metabolites (Table 4), both were associated with 1,25(OH)₂D, although the association with arsenic was not statistically significant. Mean 1,25(OH)₂D concentrations were 1.6 pg/mL higher (95% CI: 0.0, 3.3, $p = 0.05$) with a doubling of urine U concentrations, and 2.3 pg/mL higher (95% CI: -0.4, 5.1, $p = 0.09$) with a doubling of urine As concentrations. When 25(OH)D was added as a covariate to the model predicting 1,25(OH)₂D, the associations did not change (Table 4).

When stratifying by sex, the positive associations of As and U with 1,25(OH)₂D remained in girls and were weaker and non-significant in boys (p -interaction = 0.33 for As and 0.44 for U) (see Supplemental Material, Table S3), while the positive association between Tl and 25(OH)D remained in boys and was weaker and non-significant in girls (p -interaction = 0.18). The positive association between 25(OH)D and molybdenum was similar in girls and boys (p -interaction = 0.56). The only significant interaction between sex and metal exposure was for Pb and 25(OH)D, where mean 25(OH)D concentration increased 1.0 (95% CI: -0.3, 2.3) ng/mL with a doubling of BPb in girls ($p = 0.33$), while in boys, mean 25(OH)D decreased 1.4 (95% CI: -2.6, -0.1) ng/mL with a doubling of BPb ($p = 0.04$, p -interaction = 0.03) (see Supplemental Material, Table S3).

Discussion

In this cross-sectional study of 512 adolescents from a smelter town in Mexico, higher urine concentrations of Mo and Tl were associated with higher serum concentrations of 25(OH)D and higher urine concentrations of U and As were associated with higher serum concentrations of

1,25(OH)₂D. We also found that the associations of BPb and urine Cd with vitamin D metabolites were small and not statistically significant.

Although this is a cross-sectional study and the biomarkers of exposure used do not represent chronic exposure, we can infer from the participants' residential history that they have been chronically exposed, although exposures may have varied throughout their lives.

Our results were not consistent with prior studies on the association of BPb and urine Cd with vitamin D. BPb has previously been positively associated with 25(OH)D in a study of 142 African American and Hispanic children in New Jersey (Kemp et al 2006). The mean BPb in this study (4.92 µg/dL) was comparable to the mean BPb in our study. Kemp et al. collected samples across different seasons and found that both BPb and 25(OH)D concentrations were higher in the Summer compared to other seasons. It is thus possible that seasonal changes in 25(OH)D concentrations in New Jersey were associated with other factors related to Pb exposure in children, such as outside activities (Kemp et al 2006). Torreón, on the other hand, has a year-round sunny climate with less seasonal variation, making confounding by seasonality less likely, though still possible, as seen with U, when the association between U and 25(OH)D was no longer significant after adjusting for season. BPb was also negatively associated with 1,25(OH)₂D in a study of 45 African American and Puerto Rican children in New York exposed to high levels of Pb in the 1970s (Rosen et al 1980). In this study, mean BPb concentrations in the three Pb exposure groups were 18 (n = 15), 47 (n = 18) and 74 (n = 12) µg/dL, respectively (Rosen et al 1980). In contrast, the 90th percentile for blood lead concentration in our study was 7.7 µg/dL. While still above the current CDC BPb reference value for children aged 1-5 years (5 µg/dL), BPb concentrations in our study may not have been high enough to affect vitamin D metabolism.

Also in contrast to our results, a previous study reported that Cd exposure was inversely associated with $1,25(\text{OH})_2\text{D}$ (Nogawa et al 1987). In this study, Japanese men and women 59 years of age or older who had either itai-itai disease ($n = 5$) or known cadmium exposure and renal disease from living near a zinc mine ($n = 36$) had lower concentrations of $1,25(\text{OH})_2\text{D}$ compared to healthy, unexposed control participants ($n = 17$). The mean urine Cd in exposed participants ranged from 12-16 $\mu\text{g/g}$ creatinine, which is 60-80 times higher than the mean Cd concentrations in our study (0.2 $\mu\text{g/g}$ creatinine). It is possible that $1,25(\text{OH})_2\text{D}$ concentrations were decreased in Cd exposed cases because of Cd-induced kidney disease, as the hydroxylation of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ takes place in the kidney (Jones and Prosser, 2011). As with BPb, Cd concentrations in our study may have not been high enough to affect vitamin D metabolism either directly or via affectation of kidney function.

We found a significant positive association between urine U concentrations and $1,25(\text{OH})_2\text{D}$. In one prior study, rats that were administered very high doses of uranium (1 mg/rat/day, twice the highest naturally-occurring exposure on earth) had $1,25(\text{OH})_2\text{D}$ decreased by 56% compared to control rats, while $25(\text{OH})\text{D}$ was the same in both groups (Tissandie et al 2007). The high doses of U exposure in this experiment and between species variability make it difficult to compare these findings with the results of our study. We also found a significant positive association between urine As and $1,25(\text{OH})_2\text{D}$. We could not identify prior research in this area.

One possible explanation for the positive association between U or As with $1,25(\text{OH})_2\text{D}$ is that these trace elements may induce a sub-clinical Fanconi syndrome. Fanconi syndrome is characterized by damage to the renal proximal tubule that results in increased urinary excretion of various minerals including phosphorus, and may result in higher $1,25(\text{OH})_2\text{D}$ (Tieder et al, 1988). Indeed, ingestion of a high amount of U has been implicated in the development of a

partial Fanconi syndrome in a case report of an individual (Pavlakakis et al 1996). Lead exposure at higher concentrations than in our study has also been associated with the development of a Fanconi-like syndrome with proximal tubular dysfunction in 134 children from Chicago who had been treated for lead poisoning (Loghman-Adham et al 1998). Future studies with measurements of phosphorus levels are needed to confirm this hypothesis. However, while the kidney is the major source of $1,25(\text{OH})_2\text{D}$, extra-renal synthesis does occur and we cannot exclude the possibility of other organs playing a role in the associations seen in this study.

We also found significant positive associations between urine Mo and Tl with $25(\text{OH})\text{D}$. We could not find any prior studies on these associations and there is no clear mechanistic explanation linking metal exposure to increased concentrations of $25(\text{OH})\text{D}$. Since $25(\text{OH})\text{D}$ is greatly influenced by sun exposure, metal concentrations may reflect environmental exposures that occur during outside activities and may thus be associated with increased $25(\text{OH})\text{D}$. In our study, we attempted to adjust for time spent outside by asking participants about how much time they spent engaging in physical activity. None of the outdoor sports registered, including soccer, volleyball, bicycle riding, tennis, baseball, running, or swimming, predicted $25(\text{OH})\text{D}$ concentrations. The only predictor of $25(\text{OH})\text{D}$ was the amount of time spent walking. When we adjusted for time spent walking, the associations between metal exposures and $25(\text{OH})\text{D}$ did not change appreciably, but time spent walking may be a limited marker of sunlight exposure.

The strengths of this study include the use of high-quality, sensitive measurements of urinary trace elements and vitamin D metabolites, the availability of serum $1,25(\text{OH})_2\text{D}$ concentrations, not frequently measured in human epidemiological studies, and the availability of information on a number of potential confounders. Although serum $1,25(\text{OH})_2\text{D}$ is not frequently measured and serum $25(\text{OH})\text{D}$ is considered the standard biomarker for vitamin D status, $1,25(\text{OH})_2\text{D}$ is the

active form of vitamin D and is responsible for its biological activity. Future research is needed to determine if the differences in mean serum 1,25(OH)₂D concentrations estimated in association with exposure were large enough to be clinically relevant.

Some limitations need to be considered in the interpretation of our findings. Since this is the first report for several of these associations and there is limited information on potential mechanisms, it is necessary to confirm these results in independent studies. We also cannot exclude the possibility that the associations seen in this study are due to chance. Another limitation is that 25(OH)D and 1,25(OH)₂D have very different half-lives, with 25(OH)D being several weeks and 1,25(OH)₂D being several hours. It is possible that the differing half-lives affected our findings, by limiting the number of associations seen with 1,25(OH)₂D. Unfortunately, in a cross-sectional study, we cannot be sure. As with other observational designs, the cross-sectional design of this study limited our ability to establish causality. Furthermore, the cross-sectional design cannot establish the temporal sequence of the association between vitamin D and metals. It is also possible that unmeasured confounders, including genetics, which influence both trace element concentrations and vitamin D status could explain the associations. The absence of speciated arsenic measurements is another limitation of this study. In addition, smoking history was collected by self-report, and biomarkers of smoking exposure were unavailable. Self-reporting of smoking, particularly in adolescents, may be inaccurate, which is a limitation. As smoking is associated with lower concentrations of both 25(OH)D and 1,25(OH)₂D (Brot et al, 1999), inaccurate information on smoking history could bias our results. Finally, we studied a very narrow age range of participants in a smelter city, and it is unclear if our findings can be generalized to other ages or to populations in areas with different levels of environmental metal exposures.

In this study, we found that urine U, Mo, and Tl were positively associated with serum 25(OH)D and urine U and As were positively associated with serum 1,25(OH)₂D concentrations. From these results, we conclude that some non-essential trace elements may affect vitamin D metabolism, and this relationship deserves further study. Future studies should employ longitudinal designs and include measurements of calcium and phosphorus metabolism to better understand the implications of early trace element exposure on vitamin D and mineral metabolism.

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Table 1. Descriptive characteristics of study participants (N = 512).

Characteristic	Number (%) or mean \pm SD	Median	25th %ile	75th %ile
Age (years)	14.0 \pm 1.2	13.9	13.0	15.0
Male	262 (51.2)			
Monthly family income below 3000 pesos (\$230)	311 (60.7)			
Never smoker	308 (60.2)			
Past smoker	149 (29.1)			
Current smoker	55 (10.7)			
Fat mass (%)	26.4 \pm 8.8	26.0	19.1	32.3
25(OH)D (ng/mL)	24.8 \pm 8.2	24.1	19.2	29.2
1,25(OH) ₂ D (pg/mL)	58.6 \pm 18.3	55.4	44.7	69.8
BPb (μ g/dL)	4.6 \pm 2.8	4.0	3.0	5.6
Cd (μ g/g creatinine)	0.3 \pm 0.4	0.22	0.15	0.33
As (μ g/g creatinine)	41.1 \pm 26.1	36.6	26.5	47.7
U (μ g/g creatinine)	0.07 \pm 0.3	0.04	0.03	0.06
Mo (μ g/g creatinine)	70.1 \pm 34.0	63.0	48.0	83.3
Tl (μ g/g creatinine)	0.31 \pm 0.18	0.27	0.19	0.38

Table 2. Change in mean 25(OH)D (ng/mL) concentrations by doubling of exposure or quartile of exposure.

Exposure	Log₂-transformed β (95% CI)	p-value	Q1	Q2 β (95% CI)	Q3 β (95%) CI	Q4 β (95% CI)	p-trend
Pb							
Model 1	-0.2 (-1.2, 0.7)	0.67	ref	1.6 (-0.4, 3.6)	0.3 (-1.7, 2.4)	-0.1 (-2.1, 2.0)	0.70
Model 2	-0.5 (-1.4, 0.4)	0.32	ref	0.6 (-1.3, 2.4)	0.3 (-1.6, 2.2)	-0.6 (-2.6, 1.3)	0.48
As							
Model 1	0.7 (-0.4, 1.8)	0.22	ref	1.1 (-0.9, 3.1)	0.9 (-1.1, 3.0)	2.1 (0, 4.2)	0.07
Model 2	0.1 (-0.9, 1.1)	0.87	ref	0.2 (-1.6, 2.1)	-0.2 (-2.1, 1.7)	1.3 (-0.7, 3.2)	0.30
Cd							
Model 1	0.1 (-0.6, 0.8)	0.75	ref	0.5 (-1.5, 2.5)	1.3 (-0.7, 3.3)	0.7 (-1.3, 2.7)	0.37
Model 2	0.3 (-0.4, 0.9)	0.40	ref	0.7 (-1.2, 2.5)	1.6 (-0.3, 3.4)	0.6 (-1.2, 2.5)	0.37
Mo							
Model 1	2.4 (1.2, 3.5)	< 0.01	ref	1.5 (-0.5, 3.4)	1.8 (-0.2, 3.8)	3.8 (1.7, 5.8)	< 0.01
Model 2	1.5 (0.4, 2.6)	0.01	ref	0.7 (-1.2, 2.5)	0.5 (-1.3, 2.3)	2.5 (0.6, 4.4)	0.02
TI							
Model 1	1.2 (0.2, 2.2)	0.02	ref	1.2 (-0.8, 3.2)	1.1 (-0.9, 3.1)	1.9 (-0.2, 3.9)	0.10
Model 2	1.2 (0.3, 2.1)	0.01	ref	1.1 (-0.7, 2.9)	0.6 (-1.2, 2.5)	1.9 (0.0, 3.8)	0.10
U							
Model 1	0.7 (0.1, 1.4)	0.04	ref	0.1(-1.9, 2.2)	2.1 (0.1, 4.1)	2.7 (0.6, 4.7)	< 0.01
Model 2	0.2 (-0.4, 0.8)	0.50	ref	0.1 (-1.7, 2.0)	1.3 (-0.6, 3.1)	1.0 (-0.9, 2.9)	0.18

Model 1: Adjusted for age and sex.

Model 2: Adjusted for age, sex, season, SES (family income < 3000 pesos/month, ≥ 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.

P-trend was calculated by entering the exposure quartiles as a continuous variable.

The cutpoints for quartiles were as follows: Pb 2.9, 4.0, 5.6 µg/dL; As: 26.5, 36.6, 47.7 µg/g creatinine; Cd: 0.1, 0.2, 0.3 µg/g creatinine; Mo: 48.7, 63.0, 83.3 µg/g creatinine; TI: 0.2, 0.3, 0.4 µg/g creatinine; U: 0.03, 0.04, 0.06 µg/g creatinine.

Table 3. Change in mean in 1,25(OH)₂D (pg/mL) concentrations by doubling of exposure or quartile of exposure.

Exposure	Log₂-transformed β (95% CI)	p-value	Q1	Q2 β (95% CI)	Q3 β (95% CI)	Q4 β (95% CI)	p-trend
Pb							
Model 1	-0.4 (-2.5, 1.7)	0.70	ref	-0.2 (-4.6, 4.3)	-2.4 (-7.0, 2.1)	0.8 (-3.8, 5.4)	0.96
Model 2	-0.3 (-2.4, 1.9)	0.82	ref	-0.6 (-5.1, 3.9)	-1.6 (-6.2, 3.0)	1.1 (-3.7, 5.9)	0.75
Model 3	0.0 (-2.2, 2.1)	0.98	ref	-0.9 (-5.3, 3.5)	-1.7 (-6.3, 2.8)	1.4 (-3.3, 6.1)	0.64
As							
Model 1	3.6 (1.2, 6.0)	< 0.01	ref	2.5 (-1.9, 7.0)	6.0 (1.5, 10.4)	6.4 (1.8, 11.0)	< 0.01
Model 2	3.4 (0.9, 5.9)	0.01	ref	2.4 (-2.1, 6.9)	6.2 (1.7, 10.8)	5.8 (1.1, 10.5)	< 0.01
Model 3	3.3 (0.9, 5.8)	0.01	ref	2.3 (-2.2, 6.7)	6.3 (1.9, 10.8)	5.2 (0.6, 9.9)	0.01
Cd							
Model 1	-0.4 (-2.0, 1.1)	0.58	ref	-0.8 (-5.3, 3.6)	0.7 (-3.8, 5.1)	-0.2 (-4.6, 4.3)	0.65
Model 2	0.0 (-1.6, 1.6)	0.99	ref	-1.2 (-5.7, 3.3)	1.0 (-3.5, 5.5)	0.4 (-4.2, 4.9)	0.77
Model 3	-0.1 (-1.7, 1.5)	0.87	ref	-1.5 (-5.9, 2.9)	0.3 (-4.1, 4.7)	0.1 (-4.4, 4.5)	0.78
Mo							
Model 1	1.8 (-0.8, 4.4)	0.18	ref	-0.7 (-5.1, 3.8)	3.7 (-0.7, 8.2)	3.7 (-0.7, 8.3)	0.03
Model 2	1.5 (-1.2, 4.1)	0.28	ref	-1.3 (-5.8, 3.2)	3.3 (-1.2, 7.8)	3.2 (-1.4, 7.9)	0.05
Model 3	0.8 (-1.9, 3.4)	0.57	ref	-1.6 (-6.0, 2.8)	3.1 (-1.3, 7.5)	2.0 (-2.6, 6.6)	0.13
TI							
Model 1	-1.4 (-3.5, 0.8)	0.21	ref	-1.6 (-6.1, 2.8)	-3.0 (-7.5, 1.5)	-3.4 (-8.0, 1.2)	0.12
Model 2	-0.6 (-2.8, 1.6)	0.61	ref	-1.3 (-5.7, 3.2)	-2.6 (-7.1, 1.9)	-2.0 (-6.7, 2.6)	0.31
Model 3	-1.2 (-3.4, 1.0)	0.30	ref	-1.8 (-6.2, 2.5)	-2.9 (-7.4, 1.5)	-3.0 (-7.6, 1.6)	0.17
U							
Model 1	2.0 (0.5, 3.4)	0.01	ref	3.9 (-0.6, 8.3)	4.9 (0.4, 9.3)	8.1 (3.6, 12.6)	< 0.01
Model 2	2.2 (0.7, 3.7)	0.01	ref	4.7 (0.2, 9.1)	5.0 (0.5, 9.4)	8.2 (3.6, 12.9)	< 0.01
Model 3	2.1 (0.6, 3.6)	0.01	ref	4.6 (0.3, 9.0)	4.4 (0.0, 8.8)	7.8 (3.2, 12.3)	< 0.01

Model 1: Adjusted for age and sex.

Model 2: Adjusted for age, sex, season, SES (family income < 3000 pesos/month, \geq 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.

Model 3: Adjusted for age, sex, season, SES, smoking, adiposity, time spent outside, 25(OH)D.

P-trend was calculated by entering the exposure variable as a continuous variable.

The cutpoints for quartiles were as follows: Pb 2.9, 4.0, 5.6 $\mu\text{g/dL}$; As: 26.5, 36.6, 47.7 $\mu\text{g/g creatinine}$; Cd: 0.1, 0.2, 0.3 $\mu\text{g/g creatinine}$; Mo: 48.7, 63.0, 83.3 $\mu\text{g/g creatinine}$; Tl: 0.2, 0.3, 0.4 $\mu\text{g/g creatinine}$; U: 0.03, 0.04, 0.06 $\mu\text{g/g creatinine}$.

Table 4. Change in mean vitamin D concentrations per doubling of uranium and arsenic concentrations in a model adjusted for both exposures.

Exposure	25(OH)D (ng/mL)	p-value	1,25(OH)₂D (pg/mL)	p-value
As				
Model 1	0.3 (-1.0, 1.5)	0.68	2.7 (0.1, 5.4)	0.04
Model 2	-0.1 (-1.2, 1.1)	0.91	2.3 (-0.4, 5.1)	0.09
Model 3	NA	NA	2.4 (-0.3, 5.0)	0.08
U				
Model 1	0.7 (-0.1, 1.4)	0.08	1.3 (-0.3, 2.9)	0.12
Model 2	0.2 (-0.4, 0.9)	0.51	1.6 (0.0, 3.3)	0.05
Model 3	NA	NA	1.5 (-0.1, 3.1)	0.06

Model 1: Adjusted for age and sex.

Model 2: Adjusted for age, sex, season, SES (family income < 3000 pesos/month, ≥ 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.

Model 3: Adjusted for age, sex, season, SES, smoking, adiposity, time spent outside, 25(OH)D.